

University of Groningen

## Physical properties and structure of enzymatically synthesized amylopectin analogs

Ciric, Jelena; Woortman, Albert J. J.; Gordiichuk, Pavlo; Stuart, Marc C. A.; Loos, Katja

*Published in:*  
Starch-Starke

*DOI:*  
[10.1002/star.201300063](https://doi.org/10.1002/star.201300063)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2013

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Ciric, J., Woortman, A. J. J., Gordiichuk, P., Stuart, M. C. A., & Loos, K. (2013). Physical properties and structure of enzymatically synthesized amylopectin analogs. *Starch-Starke*, 65(11-12), 1061-1068. <https://doi.org/10.1002/star.201300063>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## RESEARCH ARTICLE

# Physical properties and structure of enzymatically synthesized amylopectin analogs

Jelena Ciric<sup>1</sup>, Albert J. J. Woortman<sup>1</sup>, Pavlo Gordiichuk<sup>1</sup>, Marc C. A. Stuart<sup>2</sup> and Katja Loos<sup>1</sup>

<sup>1</sup> Department of Polymer Chemistry and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands

<sup>2</sup> Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands

The mechanism of the enzymatic polymerization of amylopectin analogs with phosphorylase *b* and glycogen branching enzyme is very intriguing. Recently, size exclusion chromatography with multi-detection of enzymatically synthesized amylopectin analogs in combination with MALDI-ToF MS analysis of enzymatically debranched analogs was used to solve parts of the molecular mechanism of analog's enzymatic polymerization. In this work dynamic light scattering (DLS), AFM and cryo-TEM, respectively were used to determine structural characteristics of the same analogs. The results were compared with SEC analyses. The presented analyses in this work fully agreed with the recently made observations and confirmed the changes in the architecture of the synthesized polysaccharide due to the change of enzymatic polymerization mechanism. Furthermore, we showed that the synthetic amylopectin analogs are stable to retrogradation at 4°C if the main side chain length is no longer than 12 glucose units and that they have mostly fluid-like behavior in the form of 20% suspensions.

Received: March 4, 2013

Revised: April 3, 2013

Accepted: April 5, 2013

**Keywords:**

Amylopectin / Enzymatic synthesis / Retrogradation / Size

## 1 Introduction

The relation of synthesis-molecular architecture-properties of starch and its components is very important as starch is the most consumed carbohydrate in human nutrition. However, it is very demanding to unravel this relation as starch is difficult to characterize partly due to solubility issues, stability, degradation during size separation, the broadness of size distribution, shear scission, etc. [1, 2]. During the last decades several approaches have been used in order to establish this relation. For instance, progress of biotechnology made it possible to produce tailor-made starches in vivo and to study the resulting properties [3, 4]. Furthermore, separation of the components of starch and their character-

istics has been investigated intensively in the past [5–8]. Another approach is to study synthetic tailor-made analogs of starch components and to establish a detailed insight into the molecular architectures and properties via this route. We recently synthesized well-defined amylopectin analogues (hyper-branched amylose) successfully via an in vitro tandem reaction using the enzymes phosphorylase *b* and *Deinococcus geothermalis* glycogen branching enzyme (Dg GBE) as catalysts and glucose-1-phosphate (G-1-P) as a substrate [9]. The linear section of the amylopectin analogs were formed by the phosphorylase-catalyzed propagation of G-1-P [10, 11]. Branches at the  $\alpha$ -(1  $\rightarrow$  6) position were introduced by Dg GBE via relocation of short oligosaccharide chains from the  $\alpha$ -(1  $\rightarrow$  4) position [12, 13]. A tunable degree of polymerization was obtained by variation of the ratio between the substrate and the primer maltoheptaose (G-7), i.e., by the increase of the substrate concentration [14]. Furthermore, different average degrees of branching were achieved by the change of reaction time [9].

The biocatalytic synthesis of (branched) polysaccharides was proven to be highly successful [9, 14–16]. Such synthetic polysaccharides can be used in different fields; such as

**Correspondence:** Professor Katja Loos, Department of Polymer Chemistry and Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands  
**E-mail:** k.u.loos@rug.nl  
**Fax:** +31-50-3634400

**Abbreviations:** Dg GBE, *Deinococcus geothermalis* glycogen branching enzyme; DLS, dynamic light scattering; G-7, maltoheptaose; G-1-P, glucose-1-phosphate;  $R_h$ , hydrodynamic radius; UC, universal calibration

**Colour online:** See the article online to view Fig. 1 in color

hyperbranched glycoconjugates for drug delivery purposes; or enzymatically synthesized glycogens as anti-tumor agents [15, 17] or as standards for new characterization protocols for branched polysaccharides [17]. The complete mechanism of the tandem enzymatic polymerization with phosphorylase and branching enzyme is not known yet; nevertheless, in our previous work, we unraveled parts of the mechanism by an in-depth characterization of synthetic amylopectin analogues using SEC with multi-detection in combination with the analysis of enzymatically debranched molecules using MALDI-ToF MS [16].

In our previous work we established two groups of synthetic hyper-branched amylose – samples synthesized with different reaction time/DB; and samples synthesized with different monomer concentration/degree of polymerization. We could show that with an increase of reaction time/DB some of the linear or slightly branched amylose chains serve mainly as oligosaccharide donors for the new branches and some mainly as their acceptors during the synthesis. Simplified said, the product consists of less hyper-branched amylose molecules than primer sequences at the beginning of the reaction. Other interesting observations in this group of molecules were the existence of a two size regions (two distinct hydrodynamic radii ( $R_h$ )); a constant increase of the MW over time even after the consumption of the substrate stopped; long branches and a broad distribution of the branch lengths. Furthermore, regardless of the constant increase of the average DB, branching in the high  $R_h$  area stops after a specific reaction time and in the low  $R_h$  area branching continues.

Furthermore, we have shown that in the second group of molecules – synthesized with different monomer concentration/degree of polymerization – with increasing monomer concentration some of the short oligosaccharides cleaved by Dg GBE can serve as primers instead of becoming branches during the synthesis. As a consequence, the product consists of more hyper-branched amylose molecules than primer sequences at the beginning of the reaction. In addition, a constant decrease of the MW regardless of the used amount of substrate and the appearance of much shorter side chains were observable. The existence of two size regions is present in this group as well. Additionally, regardless of the similar average DB, branching in the low  $R_h$  area constantly decreases, whereas branching in the high  $R_h$  area is constant until a critical concentration at which it starts increasing.

To shed more light on the relation of synthesis-molecular architecture-properties of starch we studied the physical properties of samples of the extreme ends from each of the two analyzed groups. The size of the molecules was assessed by dynamic light scattering (DLS), AFM and cryo-TEM measurements. The stability of the synthetic amylopectins at 4°C was followed by DSC over 30 days. Additionally dynamic rheological measurements are presented for the same chosen samples.

## 2 Materials and methods

### 2.1 Materials

All chemicals ((G-1-P) (G7000), Tris (252859), DTT (43819), adenosine monophosphate (AMP) (A2252), phosphorylase *b* (P6635), DMSO/34869,  $\text{NaN}_3$  (S2002),  $\text{NaNO}_3$  (S5506)) were purchased from Sigma-Aldrich except LiBr (Fisher Scientific/199871000) and used without further purification. Glycogen branching enzyme from *Deinococcus geothermalis* (Dg GBE) was kindly provided by R. J. Leemhuis and L. Dijkhuizen, whereas G-7 was synthesized as reported elsewhere [14].

### 2.2 Well-defined branched polysaccharides

The selected synthetic branched polysaccharides were synthesized as reported in literature [9, 17]. G-1-P was dissolved in Tris buffer (100 mM, pH 6.7, 0.02%  $\text{NaN}_3$ ) containing G-7 (0.7 mM) as primer, DTT (1.3 mM) as reducing agent, and AMP (3.5 mM) as phosphorylase *b* activator, and the pH was adjusted to 7. The polymerization was catalyzed by addition of rabbit-muscle phosphorylase *b* (1.5 U/mL) and the branching was initiated by Dg GBE (250 U/mL) at 37°C. The reaction time was 6 or 72 h, respectively, to obtain the average DB 7 and 10–12. The concentration of G-1-P was 35–420 mM to obtain different number-average degrees of polymerization. Termination was done by a 5 min heat-treatment in a water bath. The samples were dialyzed to remove an excess of monomer, primer, and reducing agent. Subsequently, the samples were freeze-dried. Estimation of the average degree of polymerization was performed by UV-spectrometry (indirectly via quantitative determination of the liberated inorganic phosphate during the synthesis) [9], while the average DB was determined by  $^1\text{H}$  NMR as explained in literature [14]. The polysaccharides used in this study are labeled 3.4 (reaction time 6 h, ratio between monomer and the primer 300, average DB 7), 6.4 (reaction time 72 h, ratio between monomer and the primer 300, average DB 12), 6.2 (reaction time 72 h, ratio between monomer and the primer 100, average DB 12), and 6.7 (reaction time 72 h, ratio between monomer and the primer 600, average DB 10).

SEC with multi-detection analysis and MALDI-ToF MS of debranched samples were performed as specified elsewhere [17].

#### 2.2.1 SEC and MALDI-ToF MS

The SEC system set-up (Agilent Technologies 1260 Infinity) from PSS (Mainz, Germany) consisted of an isocratic pump, auto sampler without temperature regulation, an online degasser, an inline 0.1  $\mu\text{m}$  filter, an RI detector (G1362A 1260 RID Agilent Technologies), viscometer (ETA-2010 PSS,

Mainz), and MALLS (SLD 7000 PSS, Mainz). WinGPC Unity software (PSS, Mainz) was used for data processing. The samples were injected with a flow rate of 0.5 mL/min into a Suprema pre-column and three Suprema SEC columns 100, 3000, and 3000 which were also purchased from PSS. The columns and the detectors were held at 50°C. A standard pullulan kit (PSS, Mainz, Germany) with MWs from 342 to 805 000 Da was used to generate a universal calibration (UC) curve, in order to determine the hydrodynamic volume from the elution volume. The branched samples were dissolved directly in previously prepared water or 50 mM NaNO<sub>3</sub> with the addition of 0.02% NaN<sub>3</sub> in both cases in order to minimize bacterial activity, at concentration of 2 g/L. The specific RI increment value,  $dn/dc$  for the well-defined branched polysaccharides, for the calculations, in this system was taken to be the same as pullulan 0.149 mL/g, since in literature values for amylopectin vary around this value [18, 19]. The samples were mixed overnight at room temperature by thermo shaker with 350 rpm. All samples were filtered through 0.45 µm filters after shaking. Standards were dissolved in the same eluent at room temperature in a concentration of 2 g/L.

MALDI-ToF measurements were performed on a Voyager-DE PRO spectrometer in positive ion mode. 2,5-dihydroxybenzoic acid (DHB) was used as a matrix. The matrix solution was made by dissolving DHB (0.2 M) in a 1:1 v/v water/ACN solution. Sample solution was prepared by dissolving the debranched sample in water R.O. (6 g/L). Sample and matrix were mixed in the ratio 2:1 or 1:1 v/v. 0.75 µL of the mixture was pipetted on the target and left for some time to dry.

### 2.3 DLS

DLS measurements were carried out at room temperature on a ALV CGS-3 goniometer set-up equipped with a JDSU laser model 1218-2 (wavelength  $\lambda_0 = 632.8$  nm) and an ALV LSE-5005 multiple  $\tau$  digital correlator. All measurements were performed in triplo and repeated on different days to check the repeatability of the procedure. DLS measurements were performed at suitable dilutions at angles between 30° and 150° with 20° interval. The RI of the solvent (water) is  $n_0 = 1.332$ ; CONTIN algorithm was used to calculate the decay rates of the distribution functions.  $R_h$  was calculated from the diffusion coefficient, which was extrapolated to zero angle. In order to avoid dust, all samples were dissolved in filtered solvent (PTFE 0.2 µm filters Sartorius Stedim Biotech GmbH, Germany) and additionally filtered prior to analysis through PTFE 0.45 µm filters Sartorius Stedim Biotech GmbH, Germany.

### 2.4 AFM of well-defined branched polysaccharides

The sample was dissolved in filtered water, with a final concentration of 0.01 mg/mL; and deposited onto a mica

substrate. Subsequently, the substrate with the sample was dried in air for 15 min at 60°C. AFM images were recorded with a Multimode 8, controller V instrument operating in tapping mode with a SNL-10, A microcantilevers. During the measurements, the integral gain was 1.5 V, proportional gain was 5 V, and the scan rate was 1 Hz.

### 2.5 Cryo-TEM of well-defined branched polysaccharides

Aqueous sample solutions were used with a concentration of 10 g/L for the cryo-TEM. Microscopy was carried out after rapid freezing of the samples in liquid ethane (Vitrobot, FEI, Endhoven, The Netherlands) and performed on a Philips CM12 transmission electron microscope operating at an accelerating voltage of 120 kV. Images are recorded on a Gatan slow-scan CCD camera under low-dose conditions.

### 2.6 DSC measurements of well-defined branched polysaccharides

The DSC measurements were carried out on a Perkin Elmer Pyris 1 DSC instrument. The retrogradation of well-defined branched polysaccharides was monitored at 70% water content. Samples were measured in previously weighed Stainless steel large volume pans (Perkin Elmer). After the addition of R. O. water the pans were sealed and reweighed. The sample pans were heated in an oven at 120°C for 15 min. Cooled samples were stored for 1, 7, and 30 days at 4°C prior to DSC analysis. The samples were heated from 0 to 120°C at a heating rate of 10°C/min using an empty sample pan as a reference. Each measurement was done in duplicate.

### 2.7 Rheological measurements

The rheological properties of 20% suspensions from branched polysaccharide were determined with a Physica MCR 300 rheometer using a parallel plate geometry (diameter: 50 mm, gap width 1 mm) at 20°C. As to the dynamic rheological measurements, gel characterization, and linear viscoelastic region were determined by means of a strain sweep (0.1–100% strain at 1 rad/s). Subsequently, a frequency sweep (0.1–100 rad/s) was applied at 1% strain, which was well within the linear region.

Shear rate measurements were performed with the rate sweep program at a shear rate range of 0.1–100 s<sup>-1</sup>.

## 3 Results and discussion

### 3.1 DLS measurements

In our previous work [17] we studied the dependence of the  $R_h$  on the reaction parameters with SEC with multiple

detection. To perform a more in-depth study we decided to additionally use DLS for the determination of  $R_h$ . The analyzed samples possessed a very low intrinsic viscosity (around 7 mL/g) which was independent of the MW. This is in agreement with research performed by Kajiura et al. [16]. The low intrinsic viscosity suggests that the molecules behave according to hard spheres, as expected for glycogen-type molecules [20]. Only the sample with the longest side chains (6.2) had a slightly higher intrinsic viscosity compared to the others due to higher CL [16].

Due to the low and MW independent intrinsic viscosity the calculation of  $R_h$  from the SEC data with multi-detection could be deceptive [17]. Therefore, the calculation of  $R_h$  from DLS data via the Stokes–Einstein equation is much more reliable ( $R_h$  being dependent on the translational diffusion coefficient) [21]. Therefore, the results acquired with DLS measurements were used for comparison and conformation of the estimations made previously with SEC – UC.

The  $R_h$ s of the tested samples are shown in Table 1. The agreement of the  $R_h$  values measured with different techniques is quite good. For example if we compare the results for the sample 6.4 we can see a perfect agreement between DLS and SEC, both showing an  $R_h$  of 27 nm. We should however bear in mind when comparing the results from those two techniques that one value results from a batch analysis (DLS) and the other one from column separation. Higher values from DLS measurements can be explained by the fact that bigger particles scatter more strongly and give a higher impact to the statistical value [22]. Additionally, as suggested by the SEC measurements previously performed (UC), DLS showed that with an increase of the reaction time/DB the size of the polysaccharides increases and with an increase of the monomer concentration the size of the polysaccharides decreases.

### 3.2 Microscopy measurements

AFM images of the synthetic polysaccharide molecules are shown in Fig. 1. The molecules being small in height (few nm) suggests that due to water evaporation the molecules were oriented flat on the substrate [16]. AFM analysis suggests the existence of two major particle size regions and in consistence with all other techniques used so far an increase of the size of

the particles with the increase of the reaction time/DB. In addition, it becomes observable that with the increase of the monomer concentration the size of the polysaccharides decreases and particles become less uniform.

Figure 2 shows the cryo-TEM images of the synthetic polysaccharide molecules. In accordance with the AFM analysis, TEM suggests two particle size regions (low and high  $R_h$ ). The radius measured with TEM is in agreement with the  $R_h$  measured by DLS or SEC with UC curve. As already suggested by SEC analysis [17], and the DLS measurements it is obvious that with an increase of the reaction time the radius in the high  $R_h$  region increases and with an increase of the monomer concentration the radius in the high  $R_h$  region decreases. Sample 6.2 is the only analyzed sample that formed rosette-like structures, already noticed in natural glycogens, but not in synthetic analogues [16]. It is known that  $\beta$ -particles of glycogen (20–40 nm diameter) can form larger  $\alpha$ -particles by association (60–200 nm diameter) with rosette-like structure [23]. It is important to mention that the above mentioned sample had the highest MW, the longest side chains and the most broad distribution of side chains from all the samples investigated in this work. The formation of rosette-like particles could be due to the combination of those characteristics. Mostly the long side branches make the formation of  $\alpha$ -particles possible, since  $\beta$ -particles are linked via  $\alpha$ -(1  $\rightarrow$  4) glycosidic linkages into  $\alpha$ -particles [24].

### 3.3 Retrogradation at 4 °C followed by DSC

It is known for natural amylopectins that the process of retrogradation can take place in a few hours but also to over a couple of days depending on structure, concentration and temperature. When the retrogradation occurs, natural amylopectin gels reveal an endothermic peak in the region of 40–65 °C [5]. External chains of amylopectin so called A chains are responsible for the formation of crystalline lamellas and reorganization of amylopectin during retrogradation [25]. The minimum chain length necessary for crystallization is supposed to be 10, but in the presence of longer chains short oligosaccharides can co-crystallize [26]. The rate of retrogradation is dependent on the external chain lengths, being accelerated by longer chains and slowed down by the presence of short chains [27–29].

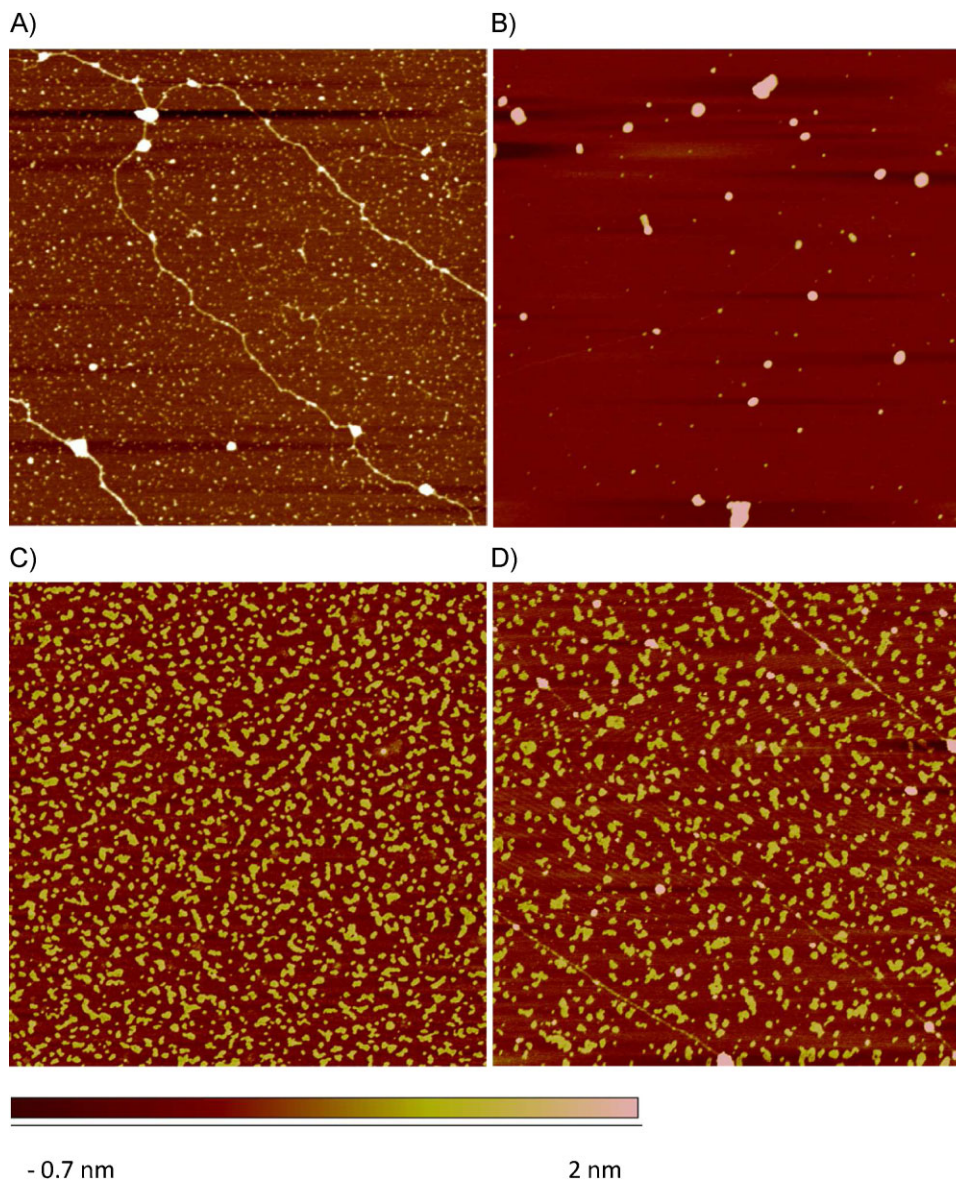
**Table 1.** Properties of the enzymatically synthesized branched polysaccharides

Sample	Time (h)	$\frac{G-1-P}{G-7}$	DP <sub>n</sub> UV <sup>a)</sup>	DB (%) <sup>1</sup> H NMR	M <sub>n</sub> (Da) SEC <sup>b)</sup> water	M <sub>w</sub> (Da) SEC <sup>b)</sup> water	$R_h$ (nm) SEC <sup>c)</sup> (UC)	$R_h$ (nm) DLS
3.4	6	300	145	700	$1.48 \times 10^5$	$7.99 \times 10^5$	≈24	12.2
6.4	72	300	192	1200	$3.46 \times 10^5$	$9.18 \times 10^5$	≈27	27.1
6.2	72	100	59	1200	$9.83 \times 10^6$	$1.07 \times 10^7$	≈30	61
6.7	72	600	342	1000	$6.09 \times 10^3$	$9.65 \times 10^5$	≈25	24.4

a) Determined via the colorimetric measurement of the liberated inorganic phosphate.

b) Determined via combination of RI and MALLS detectors.

c) Determined via UC (value taken from the SEC distribution at peak maximum).

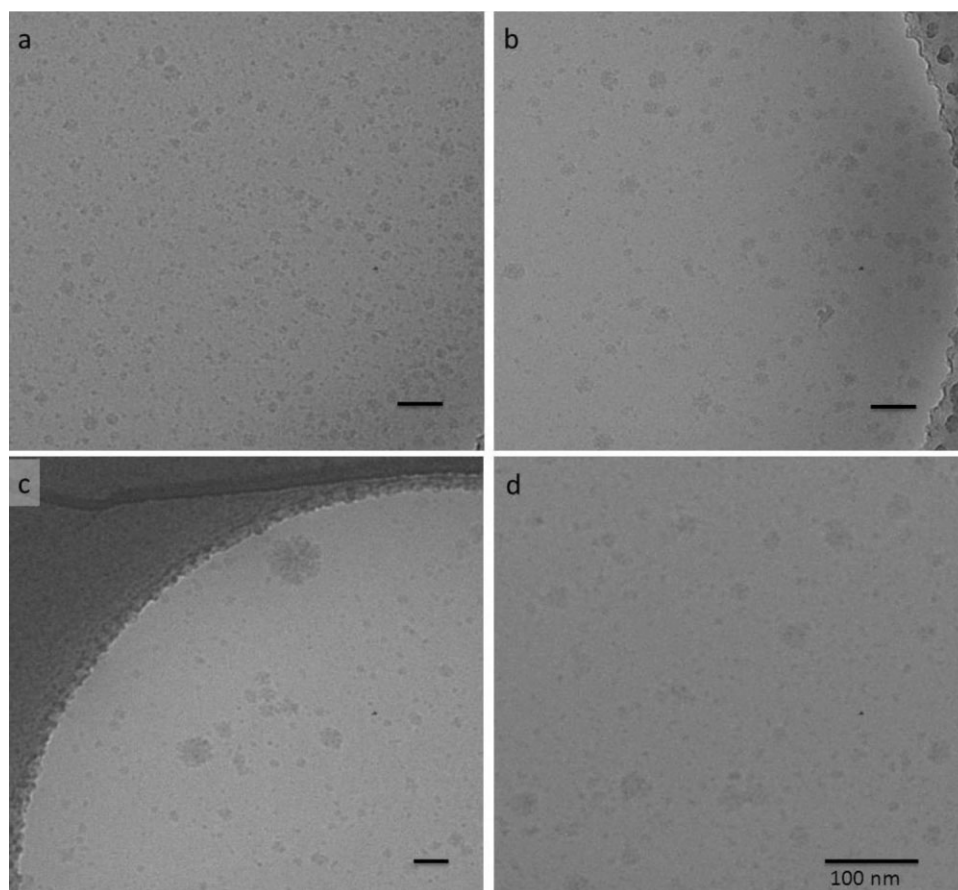


**Figure 1.** AFM images of synthetic polysaccharide (A) 3.4, (B) 6.4, (C) 6.2, (D) 6.7. Scansize 2  $\mu\text{m}$  (micrometers).

The majority of the analyzed well-defined branched polysaccharides stored at 4°C with excess water showed no presence of an endothermic peak in the monitored temperature area regardless of the storage time. This indicates that no or negligible retrogradation occurs even after 30 days.

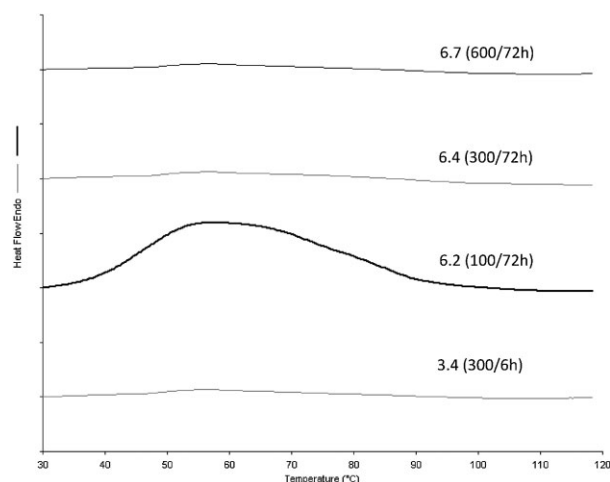
The sample 6.2 (lowest monomer concentration-longest reaction time used for the synthesis) was the only exception in this series. The endothermic peak observable in DSC was slightly broader than in the case of natural amylopectin gels (spanning 40–90°C) (Fig. 3). The broadness of the peak, when compared to literature, could be due to the different sample amounts or measuring conditions used for the experiments. The stability towards retrogradation can be explained by the absence of amylose in the analyzed suspensions as it is known that starch suspensions in water that do not contain

amylose show a lower tendency to retrogradation [30]. Additionally, retrogradation is dependent on the chain lengths of the branches. The only sample that did retrograde had the main side chain length (the most dominant chain length in the distribution) of 12 glucose units and a very broad distribution of side chains as analyzed by MALDI-ToF MS after debranching with isoamylase [17]. It is interesting that the sample 6.4 (middle monomer concentration – longest reaction time used for the synthesis) which had two main side chain lengths (two dominant chain lengths) of 9 and 12 glucose units and broad distribution of side chains [17], still showed no retrogradation. This indicates that even though the two samples (6.2 and 6.4) have similar CLs they have different external chains, different outer architecture. This interesting observation is an additional support of the proposed mechanism for the enzymatic polymerization



**Figure 2.** TEM images of synthetic polysaccharide (a) 3.4, (b) 6.4, (c) 6.2, (d) 6.7.

previously reported [17]. Due to retrogradation, sample 6.2 had a different appearance when taken out of the cold; it was a gel-crystal-like white substance, while all other samples had the same liquid opaque appearance as the suspension made at room temperature.

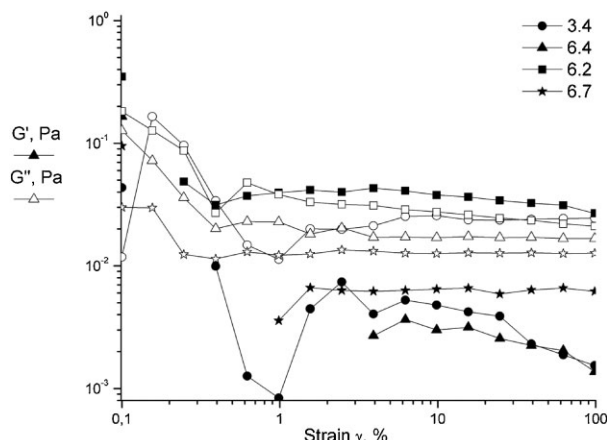


**Figure 3.** DSC heating profiles for the synthetic branched polysaccharides at 70% moisture content after storage at 4°C for 30 days.

### 3.4 Rheological study of 20% suspensions in water

Figure 4 shows the storage moduli  $G'$ , and the loss moduli  $G''$ , of all tested samples at 20% aqueous polymer concentration from the strain sweep analysis. Comparing all the samples at a given concentration, it became obvious that the only sample that possesses gel-like characteristics at this concentration is the sample 6.2 where  $G' > G''$ . The frequency sweep measurement supported the strain sweep analysis. The obtained results are a clear indication that the hydrogen bonding between the big molecules is very weak and that no association occurs. This behavior is known in modified starches in which the presence of hydroxyethyl groups disrupts the hydrogen bonding and result in fluid-like behavior [31, 32]. The weakness of hydrogen bonding cannot be explained in our case by the presence of chemical groups, but with the architecture of the polysaccharides. The longer the external chains of the molecule are, the stronger the hydrogen bonding is between the molecules. During the rheological shear rate measurements we determined the viscosity of all the analyzed samples as well. The Newtonian zone was attainable for the 20% concentrations for all the samples, which can be clearly seen in Fig. 5. The viscosities were extremely low, especially when compared to other polysaccharide solutions with that concentration [32, 33].





**Figure 4.** Dynamic strain sweeps of 20% suspensions of branched polysaccharides in water at 20°C.

## 4 Conclusions

SEC analysis prior to the experiments in this work suggested the existence of two different particles concerning size; region with high  $R_h$  and region with low  $R_h$ . With an increase of the reaction time/DB, we noticed an increase of the concentration in the high  $R_h$  region; constant increase of the average MW and a slight increase of the  $R_h$ . A combination of the presented techniques confirmed this behavior. By SEC measurements we noticed with an increase of the monomer concentration/degree of polymerization the increase of the concentration in both regions until the critical monomer concentration. When the critical monomer concentration is reached, the concentration in high  $R_h$  region starts to decrease. Unexpectedly, the MW and the  $R_h$  constantly

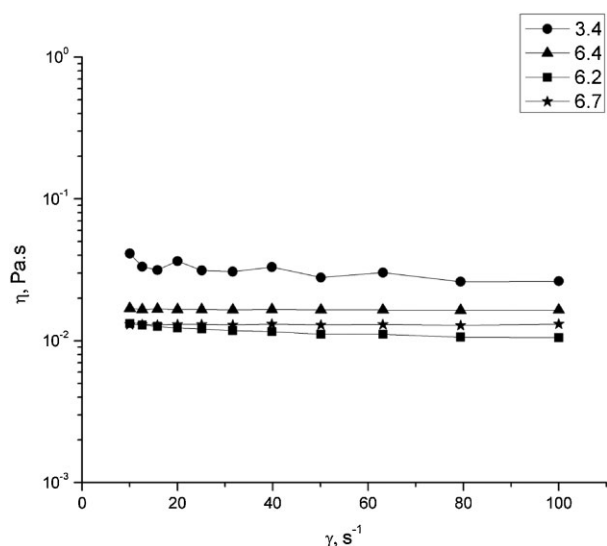
decrease. The presented analyses in this work fully agree with the observations previously made by SEC.

In this work we also showed that the synthetic amylopectin is stable to retrogradation at 4°C if the main side chain length is no longer than 12 glucose units and the polydispersity of the side chains is narrow. On the other hand we confirmed the changes in the architecture of the synthesized polysaccharide previously reported [17], due to the change of enzymatic polymerization mechanism. Fluid-like behavior of polysaccharides at the analyzed concentrations, indicate the low strength of hydrogen bonding between the chains and the absence of strong aggregation, possible due to the short chains in total or the short external chains of the molecules.

In this paper we showed interesting properties of the synthetic amylopectin, the effect of the synthesis conditions and the structure of the samples on the properties. The agreement of the particle size with different techniques such as DLS, SEC (UC), or cryo-TEM is very good. The stability towards retrogradation and low viscosity can be very interesting properties for the future application. Moreover these analysis support our previous research and suggestions made in it concerning the mechanism of the enzymatic polymerization of amylopectin analogs with phosphorylase b and Dg GBE.

*This research forms part of the research programme of the Dutch Polymer Institute (DPI), project #727. Financial support of this research by The Netherlands Organisation for Scientific Research (NWO) is greatly acknowledged. The authors would like to thank Prof. L. Dijkhuizen and Dr. R. J. Leemhuis for kindly providing Dg GBE.*

*The authors have declared no conflict of interest.*



**Figure 5.** Flow curves from 20% suspensions of branched polysaccharides in water at 20°C.

## 5 References

- [1] Gidley, M. J., Hanashiro, I., Hani, N. M., Hill, S. E. et al., Reliable measurements of the size distributions of starch molecules in solution: Current dilemmas and recommendations. *Carbohydr. Polym.* 2010, 79, 255–261.
- [2] Cave, R. A., Seabrook, S. A., Gidley, M. J., Gilbert, R. G., Characterization of starch by size-exclusion chromatography: The limitations imposed by shear scission. *Biomacromolecules* 2009, 10, 2245–2253.
- [3] Kull, B., Salamini, F., Rohde, W., Genetic engineering of potato starch composition. Inhibition of amylose biosynthesis in tubers from transgenic potato lines by the expression of antisense sequences of the gene for granule-bound starch synthase. *J. Genet. Breed.* 1995, 49, 69–76.
- [4] Kuipers, A. J., Soppe, W. J., Jacobsen, E., Visser, R. F., Factors affecting the inhibition by antisense RNA of granule-bound starch synthase gene expression in potato. *Mol. Gen. Genet.* 1995, 246, 745–755.
- [5] Durrani, C. M., Donald, A. M., Physical characterisation of amylopectin gels. *Polym. Gels Networks* 1995, 3, 1–27.



- [6] Ring, S. G., Colonna, P., l'Anson, K. J., Kalichevsky, M. T. et al., The gelation and crystallisation of amylopectin. *Carbohydr. Res.* 1987, 162, 277–293.
- [7] Kalichevsky, M. T., Jaroszkievicz, E. M., Ablett, S., Blanshard, J. M. V., Lillford, P. J., The glass transition of amylopectin measured by DSC, DMTA and NMR. *Carbohydr. Polym.* 1992, 18, 77–88.
- [8] Bizot, H., Le Bail, P., Leroux, B., Davy, J. et al., Calorimetric evaluation of the glass transition in hydrated, linear and branched polyanhydroglucose compounds. *Carbohydr. Polym.* 1997, 32, 33–50.
- [9] Ciric, J., Loos, K., Synthesis of branched polysaccharides with tunable degree of branching. *Carbohydr. Polym.* 2013, 93, 31–37.
- [10] Cori, G. T., Cori, C. F., The activating effect of glycogen on the enzymatic synthesis of glycogen from glucose-1-phosphate. *J. Biol. Chem.* 1939, 131, 397–398.
- [11] Cori, G. T., Cori, C. F., The kinetics of the enzymatic synthesis of glycogen from glucose-1-phosphate. *J. Biol. Chem.* 1940, 135, 733–756.
- [12] Fujii, K., Takata, H., Yanase, M., Terada, Y. et al., Bioengineering and application of novel glucose polymers. *Biocatal. Biotransformation* 2003, 21, 167–172.
- [13] Liu, W.-C., Castro, J. V., Gilbert, R. G., Rate coefficients for enzyme-catalyzed reactions from molecular weight distributions. *Polymer* 2011, 52, 1490–1494.
- [14] Vlist, J. v. d., Reixach, M. P., Maarel, M. v. d., Dijkhuizen, L. et al., Synthesis of branched polyglucans by the tandem action of potato phosphorylase and deinococcus geothermalis glycogen branching enzyme. *Macromol. Rapid Commun.* 2008, 29, 1293–1297.
- [15] Vlist, J. v. d., Faber, M., Loen, L., Dijkman, T. J. et al., Synthesis of hyperbranched glycoconjugates by the combined action of potato phosphorylase and glycogen branching enzyme from deinococcus geothermalis. *Polymers* 2012, 4, 674–690.
- [16] Kajiura, H., Takata, H., Kuriki, T., Kitamura, S., Structure and solution properties of enzymatically synthesized glycogen. *Carbohydr. Res.* 2010, 345, 817–824.
- [17] Ciric, J., Oostland, J., de Vries, J. W., Woortman, A. J. J., Loos, K., Size exclusion chromatography with multi detection in combination with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry as a tool for unraveling the mechanism of the enzymatic polymerization of polysaccharides. *Anal. Chem.* 2012, 84, 10463–10470.
- [18] Hoang, N.-L., Landolfi, A., Kravchuk, A., Girard, E. et al., Toward a full characterization of native starch: Separation and detection by size-exclusion chromatography. *J. Chromatogr., A* 2008, 1205, 60–70.
- [19] Yokoyama, W., Renner-Nantz, J. J., Shoemaker, C. F., Starch molecular mass and size by size-exclusion chromatography in DMSO-LiBr coupled with multiple angle laser light scattering. *Cereal Chem.* 1998, 75, 530–535.
- [20] Ioan, C. E., Aberle, T., Burchard, W., Solution properties of glycogen. 2. semidilute solutions†. *Macromolecules* 1999, 32, 8655–8662.
- [21] Helmstedt, M., Schäfer, H., Determination of sizes of spherical particles, prepared by dispersion polymerization of methyl methacrylate in non-aqueous medium, by analysis of the particle scattering and autocorrelation functions. *Polymer* 1994, 35, 3377–3383.
- [22] Putaux, J.-L., Potocki-Véronèse, G., Remaud-Simeon, M., Buleon, A.,  $\alpha$ -D-glucan-based dendritic nanoparticles prepared by in vitro enzymatic chain extension of glycogen. *Biomacromolecules* 2006, 7, 1720–1728.
- [23] Manners, D. J., Recent developments in our understanding of glycogen structure. *Carbohydr. Polym.* 1991, 16, 37–82.
- [24] Hata, K., Hata, M., Hata, M., Matsuda, K., A proposed model of glycogen particle. *J. Jpn. Soc. Starch Sci.* 1984, 31, 146–155.
- [25] Buléon, A., Tran, V., Systematic conformational search for the branching point of amylopectin. *Int. J. Biol. Macromol.* 1990, 12, 345–352.
- [26] Gidley, M. J., Bulpin, P. V., Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* 1987, 161, 291–300.
- [27] Kalichevsky, M. T., Orford, P. D., Ring, S. G., The retrogradation gelation of amylopectins from various botanical sources. *Carbohydr. Res.* 1990, 198, 49–55.
- [28] Würsch, P., Gumy, D., Inhibition of amylopectin retrogradation by partial beta-amylolysis. *Carbohydr. Res.* 1994, 256, 129–137.
- [29] Roulet, P., Macinnes, W. M., Gumy, D., Würsch, P., Retrogradation kinetics of eight starches. *Starch/Stärke* 1990, 42, 99–101.
- [30] Nuessli, J., Handschin, S., Conde-Petit, B., Escher, F., Rheology, F., Structure of amylopectin potato starch dispersions without with emulsifier addition. *Starch/Stärke* 2000, 52, 22–27.
- [31] Wurzburg, O. B. (Ed.), *Modified Starches: Properties and Uses*, CRC Press, Boca Raton, Florida, USA 1991.
- [32] Jauregui, B., Muñoz, M. E., Santamaria, A., Rheology of hydroxyethylated starch aqueous systems. Analysis of gel formation. *Int. J. Biol. Macromol.* 1995, 17, 49–54.
- [33] Tirtaatmadja, V., Dunstan, D. E., Boger, D. V., Rheology of dextran solutions. *J. Non-Newtonian Fluid Mech.* 2001, 97, 295–301.